STUDIES ON HOST-PARASITE RELATIONS OF RATS AND MICE TO *Trichosomoides crassicauda*

BY

VIVIAN SWEIBEL SMITH

A. B., Hunter College, 1940

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SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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STUDIES ON HOST-PARASITE RELATIONS OF RATS AND MICE TO *Trichosomoides crassicauda*

Trichosomoides crassicauda is a common nematode of the urinary bladder of wild and laboratory rats. It has a direct life cycle which was elucidated by Thomas in 1924. A study of host-parasite relations to a helminth living in the urinary bladder may help to shed light on complex problems of resistance to helminth infections. It may also furnish data which have a bearing on human parasites living in or around the urinary tract, in particular *Dioctophyma renale*, the giant kidney worm; and *Schistosoma haematobium*, common blood fluke of Egypt and cause of vesical schistosomiasis.

Methods

Albino rats used for laboratory studies were bred from a stock that was guaranteed parasite-free by Sprague-Dawley and Co., Madison, Wisconsin. To ensure maintenance of the colony in this condition, animals were kept in a special type of suspended cage with a removable screen bottom, and cages were autoclaved weekly throughout the experimental period.

Eggs for infection were obtained by autopsy of wild rats killed or trapped in the vicinity of Champaign and Urbana, Illinois. Gravid female worms were removed from the urinary bladder and used whole after counts had been make of the number of infective ova within or adhering to each worm. Collection of eggs from urine of infected rats was found impractical, since the eggs could not be obtained in sufficient numbers. Infecting doses were fed to animals in small meat pellets, each animal being isolated and observed until it was certain that the meat had been consumed.

Routine autopsy procedure for both wild and laboratory rats consisted of removal and detailed examination of the entire urinary tract, including kidneys, ureters, and bladder, for worms and pathology.

For serological studies, blood was collected by heart puncture and stored overnight in the refrigerator to clot. Coverglass cul-

tures of *Trichosomoides crassicauda* eggs in rat and mouse sera were prepared with semi-sterile precautions. A drop of egg suspension was added to a drop of serum within a ring of vaseline on a glass slide, and the preparation sealed with a coverglass. Cultures were incubated at 37°C.

Precipitin tests using various dilutions of rat urine vs. rat serum were of the ring type. Most readings used for final results were taken after two hours of incubation at 37°C.

In urine collections, rats were placed for eight to twelve hours in round cages resting on large funnels. A fine wire screen beneath each cage prevented passage of feces while urine was received in a vessel below.

Results

Records kept on autopsies of wild rats taken in Champaign and Urbana, Illinois, showed an average number of *Trichosomoides crassicauda* per rate of 3.5 with 65.4 percent infection. Total number of rats was 370. Difference in average number of parasites in males and females was not significant. Males averaged 3.9 worms per rat while females averaged 3.1 per rat.

Peculiar calculi found in urinary bladders of both wild and laboratory rats were established by chemical and histo-chemical tests to be composed largely of mucin. Of 32 instances of calculi encountered in wild rats, 30 were in male animals. The great majority were associated with worms in both laboratory and wild rats. However, two mucoid calculi were found in laboratory rats and two in white mice, where no previous history of infection with *T. crassicauda* existed. It is proposed that the initial stimulus for calculus formation is independent of worms in the urinary bladder, but the presence of worms provides an additional irritating factor both mechanically and chemically. Operative insertion of various substances into the urinary bladder did not induce formation of mucoid calculi.

Albino mice on balanced diets and on vitamin A-deficient diets were found to be refractory to infestion with comparatively small doses of eggs. When larvae of *T. crassicauda* were incubated in coverglass cultures in mouse serum, results were in part suggestive of the existence of a larvicidal principle in mouse serum, although further study is needed.

Small groups of young and old rats infected with 150 eggs per rat showed autopsy results indicative of the existence of an age immunity in laboratory rats. The criteria used were average number of worms and mean length of worms, both of which were reduced in old rats.

Repeated light superinfections with *T. crassicauda* gave some evidence of a degree of acquired resistance. This was more marked in animals where a series of light infections was followed by a few infections with 100 eggs. Cultures of eggs in resistant rat serum showed a striking contrast to cultures in sera of parasite-free rats. Marked precipitate-formation occurred around eggs in immune sera, but none was present in sera of animals that had never been infected with *T. crassicauda*. Animals that had been infected only once showed slight precipitate-formation which was delayed in appearance, despite the fact that parasites had not yet had time to reach the urinary tract. No precipitates formed around larvae.

Urine of animals harboring at least two mature *T. crassicauda* gave a precipitin reaction with serum of any laboratory rat regardless of previous infection. This has not been demonstrated to be a true antigen-antibody reaction and a number of complicating factors need further study and clarification. Sera from three wild rats failed to give the reaction.

Results of a brief experiment testing transmission of immunity from repeatedly infected mother rats to their offspring were not not conclusive though they were encouraging as a possible subject for future study.

Rats repeatedly infected with *T. crassicauda* did not respond to a skin test using antigen in 1:1000 dilution from a closely related parasite, *Trichinella spiralis*.

Floatation of *Trichosomoides crassicauda* eggs was attempted with saturated saline, zinc sulfate solution of specific gravity 1.18, and zinc sulfate solution of specific gravity 1.4. The first two solutions were unsatisfactory. Zinc sulfate solution of specific gravity 1.4 floated only brown-shelled eggs and those in which considerable thickening of the shell had already occurred. Other thinner-shelled eggs were distorted and fell to the bottom.

Small numbers of eggs and larvae incubated in rat serum for 36 hours (tube cultures) and injected into the peritoneal cavity of rats and mice were apparently absorbed by the tissues. Eggs incubated in artificial gastric juice, gastric juice plus bile salts, and Ringer's solution plus bile salts failed to hatch under preliminary trial conditions where pH was not adjusted.

Conclusion.

1. Calculi found in urinary bladders of rats in association with *Trichosomoides crassicauda* are largely mucoid in nature. They are in some manner linked with the male sex of host, and worms may be contributing factor but are not the sole cause of calculus-formation.

2. Diet deficient in vitamins A and D does not make mice susceptible to infection with comparatively small doses of eggs.

3. A larvicidal principle may be present in mouse serum against *T. crassicauda.*

4. Laboratory rats manifest a degree of age immunity to infection with *T. crassicauda.*

5. A low-grade acquired resistance, built up by numerous superinfections, can be induced against *T. crassicauda* in laboratory rats.

6. Precipitates are found around embryonated eggs of *T. crassicauda* that are incubated in sera of infected rats, to a degree corresponding to the amount of resistance present.

7. A precipitin-type reaction occurs between urine of rats harboring at least two mature *T. crassicauda* and sera of laboratory rats, regardless of previous history as regards infection in rats furnishing serum.

8. Rats repeatedly infected with *T. crassicauda* do not give a skin response to trichina antigen.

9. Zinc sulfate solution of specific gravity 1.4 will float mature eggs of *T. crassicauda.*

VITA

Vivian Sweibel Smith was born in New York City on October 29, 1920. She attended public schools and was graduated from Erasmus Hall High School in Brooklyn, New York in January, 1936. A New York State Regents Scholarship was received for four years of college work. At Hunter College of the City of New York, a major in biology was chosen. Following receipt of the A.B. degree in January, 1940, two academic quarters were spent at the School of Hygiene and Public Health of The Johns Hopkins University. Extensive courses in parasitology were taken.

In the summer of 1939, she attended the Marine Biological Laboratory at Woods Hole. The summer of 1940 was spent at the University of Michigan Biological Station.

Since September, 1940, she has attended the University of Illinois, holding the following positions: Scholar in Zoology, 1940-41; Assistant in Zoology, summer of 1941; Fellow in Zoology, 1941-42; Assistant in Animal Pathology and Hygiene, 1942-43; Fellow in Zoology, June, 1943 through January, 1944. The A.M. degree was received in October, 1941.

Honorary and professional societies of which she is a member include Phi Beta Kappa, Phi Sigma, Sigma Delta Epsilon, Iota Sigma Pi, Sigma Xi, and the American Society of Parasitologists.

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